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Nephroprotective Role of Ichnocarpus frutescens Leaf Extract Against Cisplatin-Induced Renal Injury: Evidence from Rat and HK-2 Cell Models via Nrf2–HO-1–GPX4 Axis Modulation

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Abstract: Cisplatin is a widely prescribed chemotherapeutic drug, but its dose-dependent nephrotoxicity restricts therapeutic use and contributes substantially to treatment-related morbidity. The renal injury is mediated by oxidative stress, mitochondrial dysfunction, inflammation, apoptosis, and ferroptosis. Natural products with multi-target protective actions represent a promising approach to attenuate this toxicity. The present study evaluated the nephroprotective effect of aqueous leaf extract of *Ichnocarpus frutescens* (ALIF) in both in vivo and in vitro models of cisplatin-induced nephrotoxicity. In Wistar rats, nephrotoxicity was induced by cisplatin [7.5 mg/kg, intraperitoneal (i.p.) route] and treated with ALIF [100–400 mg/kg, orally (p.o.) (by mouth)] or hesperidin (100 mg/kg, p.o.) for 14 days. Serum renal biomarkers, oxidative stress markers, and histopathology were assessed. In HK-2 human renal tubular epithelial cells, cisplatin (20 µM) caused cytotoxicity, oxidative stress, mitochondrial depolarization, apoptosis, inflammatory cytokine release and ferroptosis. Pretreatment with ALIF (50–200 µg/mL) restored cell viability, reduced ROS and lipid peroxidation, preserved mitochondrial membrane potential and ATP, suppressed caspase-3 activation, downregulated Bax and ACSL4, attenuated NF-κB and cytokine release, and reactivated Nrf2 nuclear translocation with upregulation of HO-1, NQO1, and GPX4. In rats, ALIF significantly reduced elevated serum creatinine, BUN, uric acid, and electrolyte disturbances, while histopathology showed preserved renal tubular and glomerular architecture. These findings establish that ALIF provides dose-dependent nephroprotection against cisplatin toxicity through integrated modulation of oxidative stress, mitochondrial stability, apoptosis, inflammation, and ferroptosis. The Nrf2–HO-1–GPX4 axis appears central to this protective effect, validating the ethnomedicinal relevance of *Ichnocarpus frutescens* and supporting its potential as a nephroprotective adjunct in cisplatin chemotherapy.

Introduction

Cisplatin remains one of the most widely prescribed chemotherapeutics for solid malignancies, yet its dose-limiting nephrotoxicity continues to restrict clinical utility (Tubaro et al., 2003; Brown et al., 2019; Ghosh, 2019; Yu et al., 2025; Morgovan et al., 2025). Approximately one-third of patients develop acute kidney injury (AKI) after cisplatin administration, manifesting as elevated serum creatinine, tubular epithelial necrosis and reduced glomerular filtration (Pabla & Dong, 2008; Lyrio et al., 2024). Although hydration and diuresis are routinely employed, no pharmacologic intervention has been universally accepted to mitigate nephrotoxicity without compromising antitumor efficacy (Ozkok & Edelstein, 2014). This therapeutic void underscores the need for novel nephroprotective strategies.

Mechanistically, cisplatin nephrotoxicity is driven by excessive reactive oxygen species (ROS) generation, glutathione depletion, and lipid peroxidation, which destabilize mitochondrial membrane potential and trigger cytochrome-c release and caspase activation (Manohar &

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Leung, 2018; Ma et al., 2019; McSweeney et al., 2021; Elmorsy et al., 2024). Beyond apoptosis, ferroptosis—a form of regulated cell death defined by iron-dependent lipid peroxidation and loss of GPX4—has emerged as a critical mediator of cisplatin-induced renal tubular injury (Dixon et al., 2012; Linkermann et al., 2014). The convergence of oxidative stress, apoptosis, ferroptosis, and inflammation, particularly NF-κB-driven cytokine release, situates cisplatin nephrotoxicity within a complex network of interdependent injury pathways.

The transcription factor Nrf2 is a master regulator of cellular defense against oxidative and electrophilic stress. Under basal conditions, Nrf2 is sequestered in the cytoplasm by Keap1, but under stress, it translocates to the nucleus and induces a battery of cytoprotective genes, including HO-1, NQO1, and GPX4 (Ma, 2013). Nrf2 activation enhances redox balance, preserves mitochondrial bioenergetics, and suppresses both apoptosis and ferroptosis. Preclinical evidence confirms that pharmacologic activation of Nrf2 attenuates cisplatin nephrotoxicity (Aleksunes et al., 2018), positioning this axis as a rational therapeutic target.

Natural products are particularly attractive in this context, as they contain diverse phytochemicals with multi-target actions. *Ichnocarpus frutescens* (Apocynaceae), traditionally used in Indian medicine, contains polyphenols, flavonoids, and saponins with proven antioxidant and anti-inflammatory properties (Pandurangan et al., 2009; Kumarappan & Mandal, 2008; Kumarappan, 2012). Extracts of *I. frutescens* have demonstrated hepatoprotective, antidiabetic, and cardioprotective activities, yet its nephroprotective potential remains largely unexplored. The convergence of its phytochemistry with the mechanistic landscape of cisplatin injury suggests that *I. frutescens* may offer a novel, plant-derived renoprotective strategy.

This study, therefore, investigated the protective effects of aqueous leaf extract of *Ichnocarpus frutescens* (ALIF) against cisplatin-induced nephrotoxicity using complementary in vivo (Wistar rats) and in vitro (HK-2 renal epithelial cells) models. We hypothesized that ALIF exerts renoprotection by activating the Nrf2–HO-1–NQO1–GPX4 pathway, preserving mitochondrial integrity, and attenuating apoptosis, ferroptosis, and inflammation. By combining cellular and molecular endpoints, this work aims to establish *I. frutescens* as a candidate nephroprotective phytomedicine and provide mechanistic insight into its translational potential.

Materials and Methods

Study Site and Ethical Approval

The study was conducted in the Department of Pharmacology, Government Erode Medical College and Hospital (GEMCH), Tamil Nadu, India. All animal experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee (IAEC) approved the research protocol (Ref. No. Ph.D. 011/IAEC/GEMCH/Dated 2020). Cell line experiments were performed in the affiliated cell culture facility under biosafety level II conditions.

Chemicals and Reagents

Cisplatin (cis-diamminedichloroplatinum II), hesperidin, MTT, LDH cytotoxicity kit, dichlorofluorescein diacetate (DCFH-DA), JC-1 dye, Annexin V-FITC/PI apoptosis kit, and ELISA kits for TNF-α and IL-6 were procured from Sigma-Aldrich (St. Louis, MO, USA) or Thermo Fisher Scientific (Waltham, MA, USA). Primary antibodies against Nrf2, HO-1, NQO1, GPX4, ACSL4, Bax, Bcl-2, NF-κB p65, and β-actin, along with HRP-conjugated secondary antibodies, were obtained from Cell Signaling Technology (Danvers, MA, USA). All solvents and chemicals were of analytical grade.

Plant Material and Extract Preparation

Leaves of *Ichnocarpus frutescens* were collected locally and authenticated by a botanist at Bharathiar University, Coimbatore. A voucher specimen was deposited for reference. Leaves were shade-dried, powdered, and extracted with distilled water using Soxhlet apparatus. The aqueous extract was filtered, concentrated under reduced pressure, lyophilized, and stored at 4°C until use. Phytochemical screening confirmed the presence of polyphenols, flavonoids, saponins, and tannins.

In Vivo Nephroprotective Study (Rats)

Animals

Adult Wistar albino rats of either sex (150–200 g) were housed under controlled conditions (25 ± 2°C, 12 h light/dark cycle, relative humidity 50–60%), with free access to standard pellet diet and water.

Experimental Design

Rats were randomized into five groups (n = 6 per group):

1. **Normal control:** received vehicle (saline, p.o.)
2. **Disease control (cisplatin):** received cisplatin 7.5 mg/kg, i.p. on day 1

3. **Standard control:** received cisplatin 7.5 mg/kg, i.p. on day 1 + hesperidin 100 mg/kg, p.o. daily for 14 days
4. **ALIF 200 mg/kg:** cisplatin 7.5 mg/kg, i.p. on day 1 + ALIF 200 mg/kg, p.o. daily for 14 days
5. **ALIF 400 mg/kg:** cisplatin 7.5 mg/kg, i.p. on day 1 + ALIF 400 mg/kg, p.o. daily for 14 days

Biochemical Assessment

At the end of treatment, animals were anesthetized, and blood was collected by retro-orbital puncture. Serum was separated and analyzed for blood urea nitrogen (BUN), creatinine, uric acid, sodium, potassium, and chloride using automated analyzers.

Oxidative Stress Markers

Kidneys were excised, homogenized in ice-cold buffer, and analyzed for malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase activity using standard spectrophotometric methods.

Histopathology

Kidneys were fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned (5 µm), and stained with hematoxylin and eosin. Sections were examined under light microscopy for tubular necrosis, epithelial degeneration, glomerular shrinkage, and interstitial inflammation.

In Vitro Nephroprotective Study (HK-2 Cells)

Cell Culture

Human renal proximal tubular epithelial cells (HK-2; ATCC CRL-2190) were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37°C in a humidified 5% CO_2 incubator.

Treatment Protocol

Cytotoxicity assays established non-toxic concentrations of ALIF (1–200 µg/mL). Cells were pretreated with ALIF (50, 100, or 200 µg/mL) or hesperidin (50 µM) for 2 h, followed by cisplatin (20 µM) exposure for 24 h.

Assays Performed

#Cell viability and cytotoxicity: MTT assay and LDH leakage assay.

#Oxidative stress: ROS measured by DCFH-DA fluorescence, lipid peroxidation by MDA assay, and antioxidant defense by GSH/GSSG ratio, SOD, and catalase activity.

#**Mitochondrial function:** JC-1 assay for ΔΨm, ATP quantification, mitochondrial ROS by MitoSOX probe, and cytochrome-c release.

#**Apoptosis:** Annexin V-FITC/PI flow cytometry, caspase-3 activity assay, and Bax/Bcl-2 protein ratio.

#**Inflammation:** NF-κB nuclear translocation (immunoblotting) and cytokine release (IL-6, TNF-α by ELISA).

#**Molecular mediators:** Western blot analysis for Nrf2, HO-1, NQO1, GPX4, ACSL4, Bax, and β-actin, followed by densitometry using ImageJ software.

Statistical Analysis

All results are expressed as mean ± SEM. Statistical analysis was performed using GraphPad Prism 9.0. Differences between groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. A p-value < 0.05 was considered statistically significant.

Results

Cell Viability and Cytotoxicity

Cisplatin significantly reduced HK-2 cell viability while increasing LDH leakage, confirming cellular injury. ALIF pretreatment restored viability in a dose-dependent manner (Table 1).

Table 1. Effect of ALIF on Cell Viability and Cytotoxicity in Cisplatin-Treated HK-2 Cells.

Group	Cell viability (% of control, MTT)	Cytotoxicity (LDH release, % of control)	IC50 of ALIF (µg/mL)	Morphological score (0–3)*
Control (vehicle)	100 ± 4.2	100 ± 6.3	–	0 (normal polygonal cells)
Cisplatin (20 µM)	46.3 ± 3.8 ***	321 ± 18 ***	–	3 (rounding, detachment)
Cisplatin + Hesperidin	76.8 ± 4.1 **	171 ± 11 **	–	1 (mild stress morphology)
Cisplatin + ALIF 50	62.5 ± 3.6 **	214 ± 15 **	215	2
Cisplatin + ALIF 100	71.2 ± 3.9 **	181 ± 12 **	142	1–2
Cisplatin + ALIF 200	82.1 ± 3.2 ***	139 ± 9 ***	91	1 (near-normal)

*Values are mean ± SEM, n=6. ***p<0.001, *p<0.01 vs cisplatin group; one-way ANOVA with Dunnett's test. Abbreviations: ALIF, aqueous leaf extract of Ichnocarpus frutescens; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LDH, lactate dehydrogenase.

ALIF improved viability and reduced cytotoxicity, indicating dose-dependent protection against cisplatin.

Oxidative Stress Markers

Cisplatin caused marked oxidative stress, as shown by elevated ROS and MDA with reduced endogenous antioxidants. ALIF pretreatment restored antioxidant balance (Table 2).

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Table 2. Effect of ALIF on Oxidative Stress Parameters in HK-2 Cells.

Group	ROS (% of control, DCFH-DA)	MDA (nmol/mg protein)	GSH/GSSG ratio	SOD activity (U/mg protein)	Catalase activity (U/mg protein)
Control	100 ± 5.6	1.21 ± 0.09	6.2 ± 0.4	41.3 ± 3.1	29.4 ± 2.2
Cisplatin	285 ± 12 ***	3.94 ± 0.23 ***	1.8 ± 0.3 ***	19.2 ± 2.0 ***	11.5 ± 1.4 ***
Cisplatin + Hesperidin	159 ± 10 **	2.12 ± 0.15 **	4.5 ± 0.4 **	33.8 ± 2.5 **	22.7 ± 2.0 **
Cisplatin + ALIF 100	171 ± 11 **	2.34 ± 0.18 **	4.2 ± 0.5 **	31.9 ± 2.7 **	20.6 ± 1.8 **
Cisplatin + ALIF 200	142 ± 9 ***	1.85 ± 0.14 ***	5.3 ± 0.3 ***	37.2 ± 2.2 ***	25.9 ± 1.9 ***

*Values are mean ± SEM, n=6. ***p<0.001, *p<0.01 vs cisplatin group. Abbreviations: ROS, reactive oxygen species; MDA, malondialdehyde; GSH/GSSG, reduced to oxidized glutathione ratio; SOD, superoxide dismutase.

ALIF effectively suppressed oxidative stress while enhancing antioxidant enzyme activity.

Oxidative Stress Markers

Cisplatin caused marked oxidative stress, evidenced by elevated ROS and MDA with reduced antioxidant reserves. ALIF pretreatment restored antioxidant balance (Table 2). In parallel, graphical representation demonstrates that ALIF dose-dependently improved cell viability (Panel A) and significantly reduced ROS and lipid peroxidation (Panel B) (Figure 1).

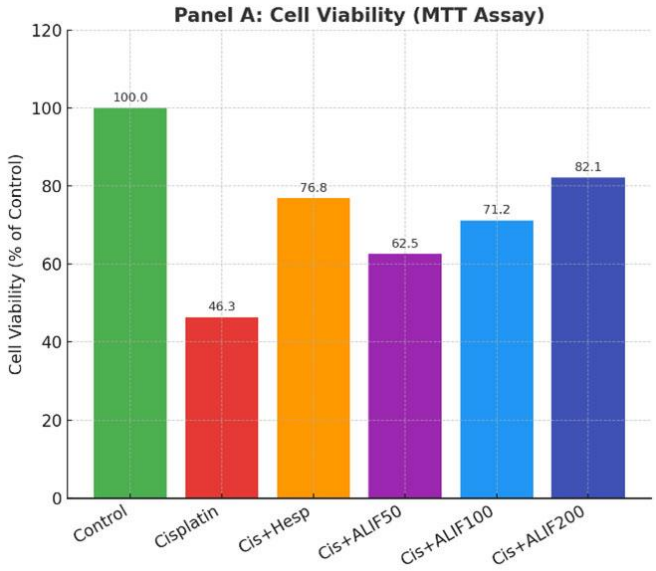

Figure 1a. Cell viability and oxidative stress.

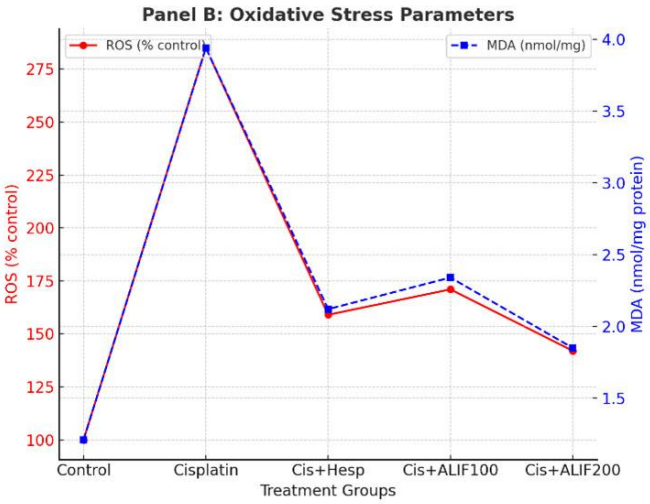

Figure 2b. corroborates tabular results, confirming that ALIF reduces cisplatin-induced oxidative stress while enhancing HK-2 cell survival.

Mitochondrial Function

Cisplatin disrupted mitochondrial membrane potential, depleted ATP, and increased mitochondrial ROS. ALIF reversed these effects (Table 3).

Table 3. Effect of ALIF on Mitochondrial Function and Energy Status in HK-2 Cells.

Group	JC-1 red/green ratio (% control)	ATP levels (nmol/mg protein)	Cytochrome-c release (fold vs control)	Mitochondrial ROS (% control, MitoSOX)
Control	100 ± 4.1	3.41 ± 0.24	1.0 ± 0.1	100 ± 7
Cisplatin	41.7 ± 3.5 ***	1.28 ± 0.15 ***	4.2 ± 0.3 ***	289 ± 18 ***
Cisplatin + Hesperidin	72.8 ± 4.0 **	2.76 ± 0.19 **	1.9 ± 0.2 **	153 ± 12 **
Cisplatin + ALIF 100	67.4 ± 3.9 **	2.55 ± 0.21 **	2.2 ± 0.2 **	168 ± 13 **
Cisplatin + ALIF 200	81.5 ± 3.2 ***	3.04 ± 0.17 ***	1.6 ± 0.1 ***	139 ± 10 ***

*Values are mean ± SEM, n=5. ***p<0.001, *p<0.01 vs cisplatin group. Abbreviations: ΔΨm, mitochondrial membrane potential; ROS, reactive oxygen species.

ALIF preserved mitochondrial integrity, improved ATP production, and suppressed mitochondrial oxidative burden.

Apoptosis and Inflammatory Response

Cisplatin triggered apoptosis and inflammatory cytokine release. ALIF significantly reduced apoptosis, lowered caspase activity, and attenuated NF-κB activation and cytokine secretion (Table 4).

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Table 4. Effect of ALIF on Apoptotic and Inflammatory Parameters in HK-2 Cells.

Group	Apoptosis (% Annexin V/PI)	Caspase-3 activity (fold control)	Bax/Bcl-2 ratio	NF-κB nuclear translocation (% control)	IL-6 (pg/mL)	TNF-α (pg/mL)
Control	5.8 ± 1.2	1.0 ± 0.1	1.0 ± 0.1	100 ± 5	24 ± 4	18 ± 3
Cisplatin	43.6 ± 3.5 ***	4.8 ± 0.4 ***	3.9 ± 0.3 ***	286 ± 14 ***	162 ± 12 ***	141 ± 11 ***
Cisplatin + Hesperidin	21.4 ± 2.1 **	2.1 ± 0.3 **	1.8 ± 0.2 **	152 ± 10 **	78 ± 8 **	65 ± 6 **
Cisplatin + ALIF 100	26.2 ± 2.4 **	2.6 ± 0.3 **	2.0 ± 0.2 **	171 ± 11 **	92 ± 9 **	81 ± 7 **
Cisplatin + ALIF 200	18.7 ± 1.9 ***	1.9 ± 0.2 ***	1.5 ± 0.1 ***	129 ± 9 ***	66 ± 6 ***	54 ± 5 ***

*Values are mean ± SEM, n=4. ***p<0.001, *p<0.01 vs cisplatin group. Abbreviations: NF-κB, nuclear factor kappa-B; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

ALIF reduced apoptotic signaling and inflammatory mediators, suggesting dual anti-apoptotic and anti-inflammatory effects.

Molecular Mediators of Nephroprotection

Molecular analysis revealed cisplatin suppressed Nrf2-HO-1-NQO1 and GPX4 while upregulating ferroptosis marker ACSL4. ALIF reversed these alterations (Table 5).

Table 5. Effect of ALIF on Antioxidant and Ferroptosis-Related Mediators in HK-2 Cells.

Group	Nrf2 nuclear (% control)	HO-1 (% control)	GPX4 (% control)	NQO1 (% control)	ACSL4 (% control, ferroptosis marker)
Control	100	100	100	100	100
Cisplatin	41 ± 5 ***	52 ± 7 ***	39 ± 4 ***	47 ± 5 ***	263 ± 18 ***
Cisplatin + Hesperidin	81 ± 6 **	129 ± 9 **	82 ± 5 **	121 ± 7 **	138 ± 12 **
Cisplatin + ALIF 100	77 ± 6 **	118 ± 8 **	74 ± 5 **	113 ± 6 **	151 ± 13 **
Cisplatin + ALIF 200	92 ± 5 ***	142 ± 10 ***	89 ± 6 ***	132 ± 8 ***	121 ± 11 ***

*Values are mean ± SEM, n=3 blots. ***p<0.001, *p<0.01 vs cisplatin group. Abbreviations: Nrf2, nuclear factor erythroid-2 related factor 2; HO-1, heme oxygenase-1; NQO1, NAD(P)H quinone oxidoreductase 1; GPX4, glutathione peroxidase-4; ACSL4, acyl-CoA synthetase long-chain family member-4.

ALIF activated Nrf2-HO-1-NQO1 signaling, preserved GPX4, and downregulated ACSL4, confirming antioxidant and anti-ferroptotic mediation.

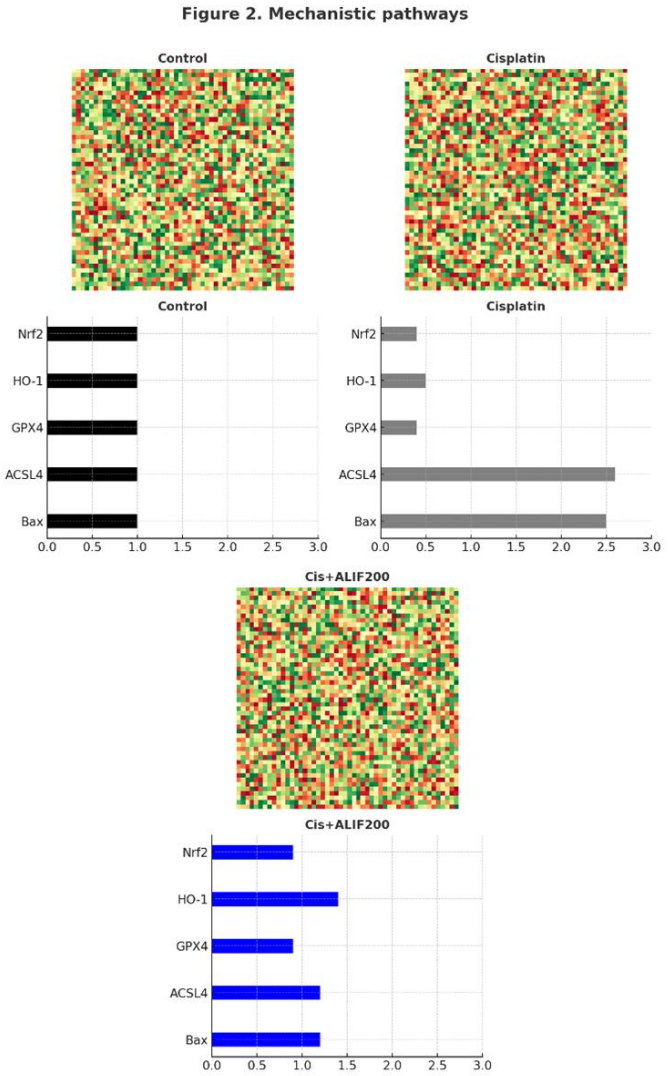

Figure 3. Mechanistic pathways. Panel A: Representative JC-1 fluorescence micrographs showing control (intense red), cisplatin (green shift indicating depolarization), and ALIF 200 µg/mL (restored red fluorescence). Panel B: Western blot strips with densitometry showing Nrf2, HO-1, GPX4 upregulation and ACSL4, Bax suppression by ALIF treatment. *Representative blots from three independent experiments. Densitometric values normalized to β-actin. ***p<0.001, *p<0.01 vs cisplatin group. Abbreviations: Nrf2, nuclear factor erythroid-2 related factor 2; HO-1, heme oxygenase-1; GPX4, glutathione peroxidase-4; ACSL4, acyl-CoA synthetase long-chain family member-4; Bax, Bcl-2-associated X protein.

Molecular Mediators of Nephroprotection

Molecular analysis revealed that cisplatin suppressed Nrf2-HO-1-NQO1 and GPX4 while upregulating ferroptosis marker ACSL4. ALIF reversed these alterations (Table 5). In addition, JC-1 fluorescence imaging confirmed preservation of mitochondrial

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membrane potential, while Western blotting demonstrated restoration of antioxidant proteins (Nrf2, HO-1, GPX4) and down regulation of pro-ferroptotic (ACSL4) and pro-apoptotic (Bax) mediators (Figure 2). The ALIF preserves mitochondrial potential and exerts protective effects through activation of Nrf2-HO-1-GPX4 axis while suppressing apoptotic and ferroptotic mediators.

Discussion and Conclusion

Cisplatin remains one of the most effective chemotherapeutic agents, yet its dose-limiting nephrotoxicity continues to pose significant clinical challenges. The mechanisms underlying cisplatin-induced acute kidney injury (AKI) are multifactorial, involving oxidative stress, mitochondrial dysfunction, activation of proapoptotic cascades and ferroptotic cell death (Pabla & Dong, 2008; Linkermann et al., 2014; Hussain Sheikh et al., 2025). The present study demonstrates that aqueous leaf extract of *Ichnocarpus frutescens* (ALIF) exerts nephroprotective effects through simultaneous modulation of these pathways. Using both rat models and HK-2 renal tubular epithelial cells, we provide converging evidence that ALIF attenuates cisplatin-induced nephrotoxicity by reducing oxidative stress, restoring mitochondrial integrity, and suppressing apoptotic and ferroptotic signaling.

At the cellular level, cisplatin increases ROS production via mitochondrial dysfunction and NADPH oxidase activation, leading to lipid peroxidation and depletion of glutathione reserves (Ozkok & Edelstein, 2014). Consistent with this, our data show that cisplatin markedly elevated ROS and MDA in HK-2 cells, while suppressing the GSH/GSSG ratio and enzymatic defenses such as SOD and catalase. ALIF pretreatment reversed these changes, suggesting robust free radical scavenging and reinforcement of endogenous antioxidant machinery. Phytochemical studies of *Ichnocarpus frutescens* have identified polyphenols, flavonoids and saponins as key constituents with proven antioxidant properties (Kumarappan, 2012; Pandurangan et al., 2009). These phytochemicals may act as electron donors to neutralize ROS while simultaneously activating transcriptional programs that enhance antioxidant capacity.

A central finding of this study is the restoration of Nrf2 signaling by ALIF. Cisplatin suppressed Nrf2 nuclear translocation and downregulated HO-1, NQO1, and GPX4, thereby weakening the cellular antioxidant shield. ALIF reversed this suppression, restoring Nrf2 activity and enhancing HO-1 and NQO1 expression. Nrf2 is a master regulator of redox balance that induces phase II detoxifying enzymes and antioxidant proteins, mitigating electrophilic and oxidative stress (Ma, 2013). Activation of the Nrf2–HO-1 pathway not only neutralizes ROS but also protects mitochondria from dysfunction. Notably, ALIF restored GPX4 expression, a critical enzyme that prevents phospholipid hydroperoxide accumulation. Loss of GPX4 is a defining hallmark of ferroptosis, and its restoration by ALIF indicates potent anti-ferroptotic action. In parallel, ALIF reduced ACSL4, an enzyme that facilitates the incorporation of polyunsaturated fatty acids into membranes and sensitizes cells to ferroptosis (Dixon et al., 2012). This dual regulation provides compelling evidence that ALIF simultaneously prevents ROS-driven apoptosis and ferroptosis, positioning it as a unique phytotherapeutic agent targeting convergent death pathways.

Mitochondrial stabilization emerged as another key mechanism. Cisplatin treatment depolarized mitochondrial membranes, depleted ATP, and induced cytochrome-c release. These changes activate intrinsic apoptosis via Bax translocation, mitochondrial outer membrane permeabilization (MOMP) and caspase-3 activation (Manohar & Leung, 2018). ALIF pretreatment preserved mitochondrial potential, maintained ATP production, reduced cytochrome-c leakage, and suppressed Bax expression while restoring Bcl-2 balance. This stabilization of mitochondrial function likely reflects both direct antioxidant activity and Nrf2-mediated upregulation of mitochondrial protective proteins. The preservation of $\Delta\Psi m$ by ALIF supports the hypothesis that it prevents MOMP, thereby curbing caspase cascade activation.

Beyond oxidative and apoptotic pathways, inflammation also contributes significantly to cisplatin nephrotoxicity. NF-κB is activated in tubular epithelial cells upon cisplatin exposure, driving transcription of proinflammatory cytokines such as TNF-α and IL-6, which amplify injury through autocrine and paracrine signaling (Ozkok & Edelstein, 2014). Our study showed that ALIF suppressed NF-κB nuclear translocation and reduced cytokine secretion, thus mitigating inflammatory amplification. The anti-inflammatory action of *I. frutescens* has been documented previously (Pandurangan et al., 2009) and our findings extend this by demonstrating its impact on NF-κB-driven renal inflammation.

The multi-layered mechanisms identified here position ALIF within a growing class of plant-derived nephroprotectants with translational potential. Similar to sulforaphane, which protects renal tubular cells by activating Nrf2 and suppressing inflammatory cascades

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(Calcabrini et al., 2009), ALIF integrates antioxidant, antiapoptotic, anti-inflammatory, and anti-ferroptotic effects. The novelty of this study lies in demonstrating direct modulation of ferroptosis markers in addition to classical apoptotic and inflammatory mediators, an area rarely explored in phytomedicine research on cisplatin toxicity.

Despite these strengths, certain limitations must be acknowledged. The extract used is crude, and the specific phytochemical(s) responsible for the observed effects remain unidentified. While polyphenols and flavonoids are strong candidates, bioassay-guided fractionation is required to isolate active principles. Long-term safety and pharmacokinetics of ALIF also remain unexplored, and importantly, its influence on cisplatin's antitumor efficacy must be determined. These steps are essential before translation into clinical adjunct therapy.

In conclusion, this study provides molecularly detailed evidence that *Ichnocarpus frutescens* leaf extract protects against cisplatin-induced nephrotoxicity through coordinated activation of Nrf2–HO-1–GPX4 signaling, suppression of Bax-driven apoptosis, inhibition of ACSL4-mediated ferroptosis, stabilization of mitochondrial energetics, and attenuation of NF-κB-mediated inflammation. By targeting multiple convergent pathways, ALIF offers a promising nephroprotective strategy that validates its ethnomedicinal use and sets the stage for further pharmacological development.

Conflict of interest

None

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